PD995 P2 - PC1

The Patent Office

PCT/GE 96 / 01388

6

The Patent Office
Cardiff Road
Newport
Gwent
NP9 1RH

PRIORITY DOCUMENT

I, the undersigned, being an officer duly authorised in accordance with Section 74(1) and (4) of the Deregulation and Contracting Out Act 1994, to sign and issue certificates on behalf of the Comptroller-General, hereby certify that annexed hereto is a true copy of the documents as originally filed in connection with the patent application identified therein.

In accordance with the Patents (Companies Re-registration) Rules 1982, if a company named in this certificate and any accompanying documents has re-registered under the Companies Act 1980 with the same name as that with which it was registered immediately before re-registration save for the substitution as, or inclusion as, the last part of the name of the words "public limited company" or their equivalents in Welsh, references to the name of the company in this certificate and any accompanying documents shall be treated as references to the name with which it is so re-registered.

In accordance with the rules, the words "public limited company" may be replaced by p.l.c., plc, P.L.C. or PLC.

Re-registration under the Companies Act does not constitute a new legal entity but merely subjects the company to certain additional company law rules.

Signed

Dated 26th June 1996

For Official use

THE PATENT OFFICE 1995

13 JUN 1995

15JUN95 E129130-1 D02029
P01/7700 25.00

ference: KR/rjp/P31202

9511935.0

Notes
Please type, or write in dark ink using CAPITAL letters. A prescribed fee is payable for a request for grant of a patent. For details, please contact the Patent Office (telephone 071-483 4700).

Rule 16 of the Patents Rules 1990 is the main rule governing the completion and filing of this form.

The Patent Office

Request for grant of a Patent

Form 1/77 **Patents Act 1977**

❶ Title of invention

1 Please give the title of the invention **NOVEL COMPOUND**

❷ Applicant's details

❑ **First or only applicant**

2a If you are applying as a corporate body please give:
Corporate Name SMITHKLINE BEECHAM PLC

Country *(and State of incorporation, if appropriate)* UNITED KINGDOM

❷ *Do not give trading styles, for example, 'Trading as XYZ company', nationality or former names, for example, 'formerly (known as) ABC Ltd' as these are not required.*

2b If you are applying as an individual or one of a partnership please give in full:

Surname

Forenames

2c **In all cases,** please given the following details:

Address: NEW HORIZONS COURT
BRENTFORD
MIDDLESEX

UK postcode *(if applicable)* TW8 9EP

Country ENGLAND

ADP number *(if known)* 05800974002 cl

Warning
After an application for a Patent has been filed, the Comptroller of the Patent Office will consider whether publication or communication of the invention should be prohibited or restricted under Section 22 of the Patents Act 1977 and will inform the applicant if such prohibition or restriction is necessary. Applicants resident in the United Kingdom are also reminded that under Section 23, applications may not be filed abroad without written permission unless an application has been filed not less than 6 weeks previously in the United Kingdom for a patent for the same invention and either no direction prohibiting publication or communication has been given, or any such direction revoked.

❹ Reference number P31202

4. Agent's or applicant's reference number *(if applicable)*

❺ Claiming an earlier application date

5. Are you claiming that this application be treated as having been filed on the date of filing of an earlier application?

Please mark correct box

Yes ☐ No ☒ ➲ *go to 6*

please give details below

☐ number of earlier application or patent number

☐ filing date *(day month year)*

☐ and the Section of the Patents Act 1977 under which you are claiming:

Please mark correct box

15(4) (Divisional) ☐ 8(3) ☐ 12(6) ☐ 37(4) ☐

❻ Declaration of priority

If you are declaring priority from a PCT Application please enter 'PCT' as the country and enter the country code (for example, GB) as part of the application number

Please give the date in all number format, for example, 31/05/90 for 31 May 1990

6. If you are declaring priority from previous application(s), please give:

Country of Filing	Priority application number *(if known)*	Filing Date *(day, month, year)*

NOVEL COMPOUND

The present invention relates to a novel compound being a novel chimeric protein, to a process for the preparation of such a compound, a pharmaceutical composition comprising such a compound and the use of such a compound in medicine, especially for the treatment of obesity and associated diseases.

European Patent Application, Publication number 0 464 533 discloses fusion proteins comprising various portions of the constant region of immunoglobulin molecules together with another human protein or part thereof. European Patent Application, Publication number 0 297 882 discloses fusion proteins comprising various portions of the plaminogen molecule with part of another human protein.

Zhang et al. (Nature: 372, 425 - 432; 1994) describe the positional cloning of a mouse obese gene and its human homologue. The sequence of the Open Reading Frame (ORF) of the mouse gene predicts a polypeptide of 167 amino acids and Zhang et al. predicted the presence of a signal sequence which would lead to the production of a mature protein of 146 residues. The human homologue was disclosed as having a similar size of 146 amino acids for the mature protein. Zhang et al. showed the presence of a primary translation product of approximate size of 18 kilodaltons (kD) with truncation to a 16kD product on addition of microsomal membranes, consistent with the production of a pre-protein and the removal of an N-terminal signal sequence. Zhang et al also disclose the potential use of the human obese gene (herein after the 'ob protein') in the treatment of obesity.

For effective, practical treatment of obesity a particularly desirable property of an obesity agent is a clearance rate in humans commensurate with patient acceptable treatment regimens, especially regimens for injectable therapies. Zhang et al. do not disclose any data concerning the physical properties of the ob protein, other than information on its apparent molecular weight as determined by SDS-PAGE. In particular, there is no disclosure relating to the clearance rate of the active molecule in either mouse or humans.

We have now discovered certain chimeric derivatives of the ob protein which have retarded clearance rates and hence are indicated to be useful for effective, practical treatment of obesity, particularly for administration by injection. These compounds are also considered to be useful for the treatment of diseases associated with obesity, such as atherosclerosis, hypertension and, especially, Type II diabetes.

and the hinge region including cysteine residues contributing to inter-heavy chain disulphide bonding, for example residues 8 and 11 of the IgG4 hinge region (Pinck J.R. and Milstein C., Nature vol216pp941-942, 1967). Preferably the IgG4 component consists of amino acids corresponding to residues 1-12 of the hinge, 1-110 of CH2 and 1-107 of CH3 of IgG4 described by Ellison J., Buxbaum J. and Hood L., DNA vol1pp11-18, 1981. In one example of a suitable mutation in IgG4, residue 10 of the hinge (residue 241, Kabat numbering) is altered from serine (S) in the wild type to proline (P) and residue 5 of CH2 (residue 248, Kabat numbering) is altered from leucine (L) in the wild type to glutamate (E).

DNA polymers which encode mutants or variants of the human immunoglobulin may be prepared by site-directed mutagenesis of the cDNA which codes for the required protein by conventional methods such as those described by G. Winter *et al* in Nature 1982, **299**, 756-758 or by Zoller and Smith 1982; Nucl. Acids Res., **10**, 6487-6500, or deletion mutagenesis such as described by Chan and Smith in Nucl. Acids Res., 1984, **12**, 2407-2419 or by G. Winter *et al* in Biochem. Soc. Trans., 1984; **12**, 224-225 or polymerase chain reaction such as described by Mikaelian and Sergeant in Nucleic Acids Research, 1992, **20**, 376.

In a further aspect, the invention provides a process for preparing a compound according to the invention which process comprises expressing DNA encoding said compound in a recombinant host cell and recovering the product.

The DNA polymer comprising a nucleotide sequence that encodes the compound also forms part of the invention.

The process of the invention may be performed by conventional recombinant techniques such as described in Maniatis *et. al.*, Molecular Cloning - A Laboratory Manual; Cold Spring Harbor, 1982 and DNA Cloning vols I, II and III (D.M. Glover ed., IRL Press Ltd).

In particular, the process may comprise the steps of:

i) preparing a replicable expression vector capable, in a host cell, of expressing a DNA polymer comprising a nucleotide sequence that encodes said compound;

ii) transforming a host cell with said vector;

iii) culturing said transformed host cell under conditions permitting expression of said DNA polymer to produce said compound; and

iv) recovering said compound.

The invention also provides a process for preparing the DNA polymer by the condensation of appropriate mono-, di- or oligomeric nucleotide units.

molecule encoding a said ob protein or variant and a second DNA molecule encoding a said immunoglobulin domain or fragment thereof.

The DNA molecules may be obtained by the digestion with suitable restriction enzymes of vectors carrying the required coding sequences or by use of polymerase chain reaction technology.

The precise structure of the DNA molecules and the way in which they are obtained depends upon the structure of the desired product. The design of a suitable strategy for the construction of the DNA molecule coding for the compound is a routine matter for the skilled worker in the art.

The expression of the DNA polymer encoding the compound in a recombinant host cell may be carried out by means of a replicable expression vector capable, in the host cell, of expressing the DNA polymer. The expression vector is novel and also forms part of the invention.

The replicable expression vector may be prepared in accordance with the invention, by cleaving a vector compatible with the host cell to provide a linear DNA segment having an intact replicon, and combining said linear segment with one or more DNA molecules which, together with said linear segment, encode the compound, under ligating conditions.

The ligation of the linear segment and more than one DNA molecule may be carried out simultaneously or sequentially as desired.

Thus, the DNA polymer may be preformed or formed during the construction of the vector, as desired.

The choice of vector will be determined in part by the host cell, which may be prokaryotic, such as *E. coli*, or eukaryotic, such as mouse C127, mouse myeloma, chinese hamster ovary or Hela cells, fungi e.g. filamentous fungi or unicellular yeast or an insect cell such as Drosophila. The host cell may also be a transgenic animal. Suitable vectors include plasmids, bacteriophages, cosmids and recombinant viruses derived from, for example, baculoviruses, vaccinia or Semliki Forest virus.

The preparation of the replicable expression vector may be carried out conventionally with appropriate enzymes for restriction, polymerisation and ligation of the DNA, by procedures described in, for example, Maniatis et al., cited above. Polymerisation and ligation may be performed as described above for the preparation of the DNA polymer. Digestion with restriction enzymes may be performed in an appropriate buffer at a temperature of 20°-70°C, generally in a volume of 50µl or less with 0.1-10µg DNA.

intake or increase in metabolic rate or oxygen consumption. Multiple injections of the fusion protein - at most twice daily - over a suitable period such as a month for primates, also causes a reduction in body weight and in the size of discrete adipose tissue deposits.

Clearance rates are determined by conventional plasma assay using ob-antibodies, for example ELISA methodology.

As indicated above the compounds of the present invention have useful pharmaceutical properties, in particular anti obesity activity and also for the treatment of diseases associated with obesity, such as atherosclerosis, hypertension and, especially, Type II diabetes.

The invention therefore further provides a pharmaceutical composition comprising a compound of the invention and a pharmaceutically acceptable carrier.

In use the compound will normally be employed in the form of a pharmaceutical composition in association with a human pharmaceutical carrier, diluent and/or excipient, although the exact form of the composition will depend on the mode of administration. The compound may, for example, be employed in the form of aerosol or nebulisable solution for inhalation or sterile solutions for parenteral administration.

The dosage ranges for administration of the compounds of the present invention are those to produce the desired anti-diabetic effect. The dosage will generally vary with age, extent or severity of the medical condition and contraindications, if any. The unit dosage can vary from less than 1mg to 300mg, but typically will be in the region of 1 to 20mg per dose, in one or more doses, such as one to six doses per day, such that the daily dosage is in the range 0.02-40mg/kg.

Compositions suitable for injection may be in the form of solutions, suspensions or emulsions, or dry powders which are dissolved or suspended in a suitable vehicle prior to use.

Fluid unit dosage forms are prepared utilising the compound and a pyrogen-free sterile vehicle. The compound, depending on the vehicle and concentration used, can be either dissolved or suspended in the vehicle. Solutions may be used for all forms of parenteral administration, and are particularly used for intravenous infection. In preparing solutions the compound can be dissolved in the vehicle, the solution being made isotonic if necessary by addition of sodium chloride and sterilised by filtration through a sterile filter using aseptic techniques before filling into suitable sterile vials or ampoules and sealing. Alternatively, if solution stability is adequate, the solution in its sealed containers may be sterilised by autoclaving. Advantageously additives such as

No unexpected toxicological effects are expected when compounds of the invention are administered in accordance with the present invention.

The following Example illustrates the invention but does not limit it in any way.

Example 1.

Construction of DNA coding for fusion protein ob 1-167/IgG4 hinge-CH2-CH3 PE variant

The gene coding for a fusion protein comprising the human 'ob' protein and the Hinge-CH2-CH3 region of human IgG4 PE (a form of IgG4 mutated as below) is created by recombinant DNA technology, preferably by a two-step recombinant PCR method.

The cDNA coding for the complete human 'ob' protein, amino acids 1-167(numbering as Y. Zhang, R. Proenca, M. Maffei, M. Barone, L. Leopold & J. Friedman. Nature 372: 425-432) is joined at the 3' end to the 5' end of the hinge-CH2-CH3 region of the cDNA coding for the human IgG4 (PE variant) protein, shown as amino acids 168-396 in the protein sequence below.

The human 'ob' gene has been prepared synthetically based on the amino acid sequence of Zhang et al, and assembled in the pcDNA3 vector. The encoded protein sequence is given in Table 2.

Human IgG4 heavy chain PE variant. In IgG4 PE, residue 10 of the hinge (residue 241, Kabat numbering) is altered from serine (S) in the wild type to proline (P) and residue 5 of CH2 (residue 248, Kabat numbering) is altered from leucine (L) in the wild type to glutamate (E). Angal S., King D.J., Bodmer M.W., Turner A., Lawson A.D.G., Roberts G., Pedley B. and Adair R., Molecular Immunology vol30pp105-108, 1993, describe an IgG4 molecule where residue 241 (Kabat numbering) is altered from serine to proline. This change increases the serum half-life of the IgG4 molecule.

The IgG4 PE variant was created using PCR mutagenesis on the synthetic human IgG4 heavy chain cDNA. The sequence of the IgG4 PE variant is described in Table 1. The residues of the IgG4 nucleotide sequence which were altered to make the PE variant are as follows:
referring to Table 1:

GACGGaTCCTTCTTCCTCTACAGCAGGCTAACCGTGGACAAGAGCAGGTGGCAGGAGGGG
900
AATGTCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAACCACTACACACAGAAGAGC
960
CTCTCCCTGTCTCTGGGTAAATGA
984

Table 2: Amino acid sequence of ob 1-167/IgG4 hinge-CH2-CH3 PE variant

SEQ ID No: 2

1 MHWGTLCGFL WLWPYLFYVQ AVPIQKVQDD TKTLIKTIVT RINDISHTQS

51 VSSKQKVTGL DFIPGLHPIL TLSKMDQTLA VYQQILTSMP SRNVIQISND

101 LENLRDLLHV LAFSKSCHLP WASGLETLDS LGGVLEASGY STEVVALSRL

151 QGSLQDMLWQ LDLSPGCESK YGPPCPPCPA PEFEGGPSVF LFPPKPKDTL

201 MISRTPEVTC VVVDVSQEDP EVQFNWYVDG VEVHNAKTKP REEQFNSTYR

251 VVSVLTVLHQ DWLNGKEYKC KVSNKGLPSS IEKTISKAKG QPREPQVYTL

301 PPSQEEMTKN QVSLTCLVKG FYPSDIAVEW ESNGQPENNY KTTPPVLDSD

351 GSFFLYSRLT VDKSRWQEGN VFSCSVMHEA LHNHYTQKSL SLSLGK